BD Vacutainer® Barricor™ Blood Collection Tube is the Tube of Choice for LC-MS/MS Analysis of Bioactive Cannabinoids in Plasma

ABSTRACT

The type of blood collection tube may influence the pharmaceutical analysis of bioactive cannabinoids in plasma. In turn, assay interference resulting from blood collection tube components can confound the interpretation of plasma pharmacokinetic and pharmacodynamics studies. We compared the new BD Vacutainer® Barricor™ Tube with five other BD blood collection tubes (BD Vacutainer® Plasma Tube with Lithium Heparin, BD Vacutainer® PST™ Tube, BD Vacutainer® Plasma Tube with Glycolytic Inhibitor, BD Vacutainer® SST™ Tube, and BD Vacutainer® Serum Tube) for plasma cannabinoid analytical within- and between-tube type accuracy and precision relative to a control (Eppendorf® LoBind microcentrifuge tube). Blank human plasma samples were fortified at three quality control concentrations and stored at room temperature for 6 h. Samples were subsequently processed for LC-MS/MS analysis. The BD Vacutainer® Barricor™ Tubes demonstrated analytically acceptable performance for all three bioactive cannabinoids evaluated in this study. Relative to the other tubes, the Barricor™ tubes showed the most consistent within- and between-run accuracy and precision relative to control. This study confirms the use of BD Vacutainer® Barricor™ Tubes for LC-MS/MS determination of plasma bioactive cannabinoid levels.

INTRODUCTION

The type of blood collection tube is a known pre-analytical factor that may influence the pharmaceutical analysis of plasma samples. Assay interference resulting from blood collection tube components, in turn, will confound the interpretation of plasma pharmacokinetic and pharmacodynamics studies. The development and validation of pharmaceutical analytical methods, then, should consider an evaluation of blood collection tubes to ensure compatibility of the analyte(s) with the blood collection tube intended for clinical trial.

BD Life Sciences – Preanalytical Systems manufactures a range of blood collection tubes for collecting, transporting, and processing of blood for testing serum, plasma, or whole blood in the clinical laboratory. An array of serum and plasma blood collection tubes is available both with and without a gel separator. The gel separator provides a barrier between the clot/cells and the serum or plasma sample, allowing primary tube sampling, transport and storage, eliminating the need to aliquot the sample.

BD offers the BD Vacutainer® Barricor™ Plasma Blood Collection Tube (BD Barricor™) with a novel non-gel separator to improve sample quality, stability and centrifugation time, enable

zinc testing, eliminate gel-related assay failures, such as instrument probe clogging, and eliminate test interferences due to gel adsorption (e.g., some therapeutic drug assays). The product is a plastic evacuated blood collection tube with a sterile interior, and a safety-engineered BD Hemogard™ closure with a low zinc stopper. The interior contains a surface coating of lithium heparin anticoagulant to prevent blood coagulation. Tube stopper and mechanical separator are lubricated with silicone based surfactant to facilitate product assembly.

OBJECTIVE

Conduct a comparative evaluation of BD Vacutainer® Barricor™ and five other BD blood collection tubes for cannabinoid LC-MS/MS within- and between-tube type accuracy and precision relative to control to identify the most appropriate blood collection tube for pharmaceutical analysis of bioactive cannabinoids in plasma samples.

MATERIALS AND METHODS

We obtained human plasma from the Canadian Blood Service with University of Saskatchewan Human Biomedical Ethics approval. We tested each human plasma source prior to experimental use to ensure absence of detectable cannabinoids in the sourced plasma. Plasma was fortified at three different quality control concentrations (Low Quality Control (LQC) = 2.53 ng/mL, Medium Quality Control (MQC) = 50.6 ng/mL, and High Quality Control (HQC) = 101.2 ng/mL) with the bioactive cannabinoids, cannabidiol (CBD), Δ^9-tetrahydrocannabinol (THC), and 11-hydroxy-Δ^9-tetrahydrocannabinol (OH-THC) (Figure 1). Fortified plasma was transferred into each of the BD Vacutainer® tubes and an Eppendorf® LoBind microcentrifuge tubes (as control) in replicates of six. All tubes were inverted eight times after transfer to ensure complete mixing.

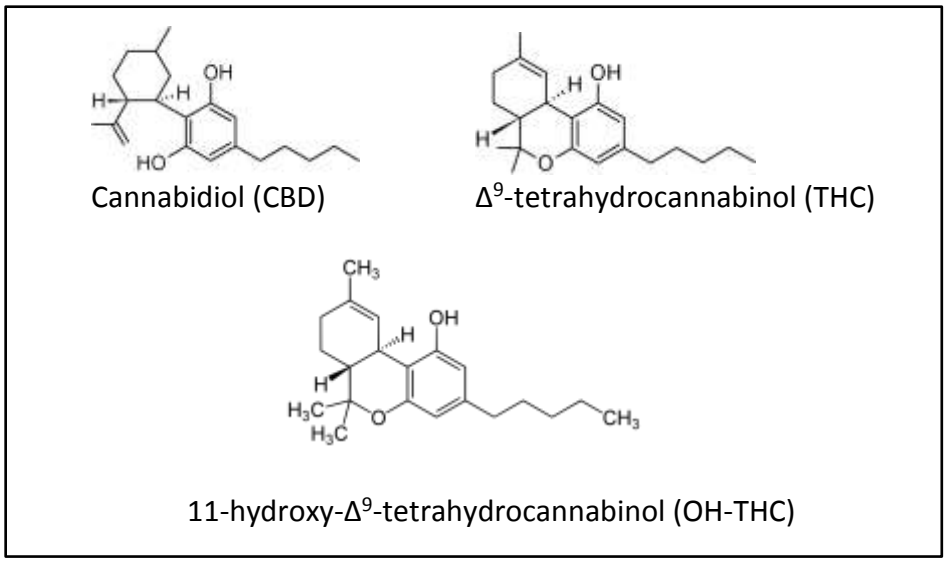

Figure 1. Chemical structures of the tested cannabinoids

All plasma samplers were incubated on bench-top for six hours at room temperature (22 ± 2°C).

After the incubation period, fortified plasma was transferred from the blood collection tubes into Eppendorf® LoBind microcentrifuge tubes. Internal standard and acetonitrile (to precipitate protein) was added and vortex-mixed for eight seconds. Samples were then centrifuged at 14000 rpm at 4°C for ten minutes. The supernatant was aliquoted into amber autosampler vials with inserts and subsequently analyzed by LC-MS/MS (Agilent® HPLC System and SCIEX® QTRAP 6500 with Turbo Spray ESI). Standard curves (R2 >0.998) were constructed according to our established analytical method based on peak area ratios of analyte to internal standard.

DATA ANALYSIS

Mean and standard deviation of all replicate samples for each quality control sample were calculated. Means were compared to control to determine percent accuracy while precision was determined as the coefficient of variation % (CV%) of replicate samples. Experimental data was analyzed using one-way analysis of variance (ANOVA) to determine the mean bias and 95% confidence limits for the between-tube comparison for each tested cannabinoid. For within-tube analysis, a one-way ANOVA as well as Tukey's Post Hoc test was conducted.

RESULTS AND DISCUSSION

A number of preanalytical factors may influence the determination of cannabinoid levels in blood. One important factor is the choice of the blood collection, a factor that does not often receive consideration prior to the conduct of pharmacokinetic and pharmacodynamic clinical studies. We conducted a comparative analysis of 6 different BD blood collection tubes to identify the tube that gave the least analytical interference or inconsistency upon LC-MS/MS analysis. To allow independent assessment of tube performance we employed a control comparator tube, Eppendorf® LoBind microcentrifuge tube, which is specifically marketed to ensure low analyte binding and minimal analytical assay interference. Furthermore, preanalytical factors might have greater affects at low or high analyte concentrations and therefore our study design included replicate assessments of low, medium, and high quality control samples to identify this potential issue.

Our data indicate quite variable results for the different blood collection tubes (Table 1). The BD Vacutainer® SST™ tube (serum, with silicone gel separator) gave the worst accuracy and precision for all three bioactive cannabinoids. The BD Vacutainer® SST™ tube was statistically different from control for CBD, THC, and OH-THC at LQC, MQC, and HQC. All other blood collection tubes demonstrated similar accuracy and precision with control at LQC for all three cannabinoids. Interestingly, for MQC, only the BD Vacutainer® Barricor™ tube and BD Vacutainer® plasma tube with glycolytic inhibitor gave similar accuracy and precision compared with the control tube while the remaining tubes were statistically different from control for CBD and THC. For HQC, the BD Vacutainer® Barricor™ tube, BD Vacutainer® plasma tube with lithium heparin (plasma, no silicone gel separator), and BD Vacutainer® plasma tube with glycolytic inhibitor (plasma, no silicone gel separator) displayed consistent accuracy and precision results to the control tubes for CBD and THC. For OH-THC only the BD Vacutainer®

Barricor™ tube and BD Vacutainer® plasma tube with lithium heparin gave consistent results to control. At all quality control concentrations for each of the three bioactive cannabinoids only the BD Vacutainer® Barricor™ tube gave consistent accuracy and precision results with no statistically significant differences relative to the control tube.

CONCLUSION

BD Vacutainer® Barricor™ Tubes demonstrated analytically acceptable performance for all three bioactive cannabinoids in this study. BD Vacutainer® Barricor™ Tubes also demonstrated analytically acceptable performance for within-tube analysis. Collection tubes that contain silicone gel separators should not be used to collect specimens for the evaluation of bioactive cannabinoids.

ACKNOWLEDGEMENT

We thank BD Life Science for the donation of BD Barricor™ tubes for evaluation in this study.

Table 1. Relative accuracy and precision of replicate (n = 6) determinations of cannabidiol (CBD), Δ^9-tetrahydrocannabinol (THC), and 11-hydroxy-Δ^9-tetrahydrocannabinol (OH-THC) fortified into blank plasma at low (LQC), medium (MQC), and high (HQC) quality control concentrations in different blood collection tubes. Accuracy was compared against the control tube (Eppendorf®) and precision expressed as a percent coefficient of variation (CV%).

		LQC		MQC		HQC	
		Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)
CBD	Eppendorf®	100	3.5	100	5.7	100	8.1
	BD Barricor™	93.4	4.6	92.6	7.4	94.7	4.8
	BD Lithium Heparin	98.7	4.5	90.5*	7.0	92.0	3.7
	BD PST™	94.8	6.1	81.8*	5.2	86.2*	4.1
	BD Glycolytic Inhibitor	96.3	5.8	94.3	4.2	91.0	5.4
	BD SST™	77.0*	20.6	81.5*	9.1	68.8*	12.5
	BD Serum	91.1	3.8	90.5*	3.2	80.0*	7.1
THC	Eppendorf®	100	4.3	100	4.6	100	6.7
	BD Barricor™	97.3	2.5	93.9	8.5	94.7	4.0
	BD Lithium Heparin	98.9	5.4	90.4*	6.0	92.0	4.7
	BD PST™	99.7	4.1	81.7*	4.6	86.3*	3.6

	BD Glycolytic Inhibitor	98.6	5.8	92.3	3.7	90.0	5.4
	BD SST™	80.1*	22.6	80.7*	9.4	78.5*	10.2
	BD Serum	94.1	1.7	90.2*	4.0	87.7*	7.6
OH-THC	Eppendorf®	100	6.7	100	4.7	100	8.7
	BD Barricor™	97.2	6.0	95.2	7.0	96.2	5.6
	BD Lithium Heparin	97.7	6.9	93.8	5.4	92.0	4.5
	BD PST™	93.5	8.2	84.1*	7.12	85.0*	3.9
	BD Glycolytic Inhibitor	93.8	6.3	93.8	3.29	86.2*	7.0
	BD SST™	77.8*	24.4	82.0*	10.7	-	-
	BD Serum	94.0	5.4	93.1	4.15	-	-

*Statistically different from control.

Authors:

Stephanie Vuong[1], Deb Michel[1], Jane Alcorn[1,2], Richard Huntsman[2,3], Richard Tang-Wai[2,4], Andrew W. Lyon[2,5].

[1] College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK, Canada.
[2] Cannabinoid Research Initiative of Saskatchewan (CRIS), University of Saskatchewan.
[3] Division of Pediatric Neurology, Department of Pediatrics, University of Saskatchewan, Saskatoon, Saskatchewan
[4] Division of Pediatric Neurology, Department of Pediatrics, University of Alberta, Edmonton, Alberta, Canada.
[4] Department of Pathology and Laboratory Medicine, Saskatoon Health Region, Saskatoon, SK, Canada.

Corresponding Author:
Dr. Andrew W. Lyon
Dept. Pathology and Laboratory Medicine
St Paul's Hospital
1702 20th Street W
Saskatoon, SK, Canada S7M 0Z9

T: 01-306-655-5164
F: 01-306-655-5232
E: Andrew.lyon@saskatoonhealthregion.ca